# Glucosepane: A New Biomarker of the Severity of Osteoarthritis



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Ρ U E. Glycation, oxidation and nitration of proteins are reactions involved in accelerated aging of tissues. The products of  $\mathbf{O}$ these reactions are used as biomarkers of chronic pathologies such as diabetes or chronic inflammatory states. In this work, we studied by mass spectrometry the levels of amino acids and glycated, oxidized or nitrated proteins in culture media of chondrocytes cultivated in multi-layers and in the blood of guinea pigs

## or osteoarthritis patients.

#### M E н Ο Sixty 3-week old male Dunkin-Hartley guinea pigs were used in this work. At 4-week old and 8-week intervals until week D **S**. 36, twelve animals were sacrificed and histological severity of knee osteoarthritis and cartilage rheological properties evaluated. Human patients with early and advanced osteoarthritis and healthy subjects were recruited. Human chondrocytes cultured in multilayers were treated for 10 days with interleukin (IL) -1 β. Amino acids and glycated, oxidized and nitrated proteins were analyzed in the serum of guinea pigs, osteoarthritis patients and in the culture medium conditioned by chondrocytes by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry using the Acquity TM UPLC system.



Age (Weeks)

### Fig 2 In vivo: Glucosepane and dityrosine increased progressively from weeks 20 and 28, respectively.

	Thickness				Young's modulus			
Biomarkers	Emoral condulas		Tibial plateau		Femoral condules		Tibial plateau	
	Correlation coefficient r	p-value	Correlation coefficient r	p-value	Correlation coefficient r	p-value	Correlation coefficient r	p-value
Glycation products								
FL							0,30	0,031
CMA					- 0,32	0,017		
Glucosepane					0,52	<0,0001*	0,56	<0,0001*
Oxydation products	3							
AASA					0,27	0,043	0,40	0,004
GSA	- 0,29	0,033	- 0,33	0,015			0,35	0,013
Dityrosine					0,34	0,010	0,36	0,010
NFK					0,37	0,006	0,33	0,018
Nitration products								
3-NT	0,33	0,013	0,29	0,034	- 0,46	0,0004*	- 0,41	0,003*
Others								
Нур	0,47	0,0003*	0,39	0,003*	- 0,38	0,0037		
CP	·			,	- 0,53	<0,0001*	- 0,33	0,018

### Fig. 1

In vitro: IL-1ß increased the concentration of the glycation, oxidation and nitration products.

		Global histological score	
		r	p-value
Biomarkers			
Glycation products			
	FL	0,33	0,012
	CEL	0,43	0,0007
	3DG-H	0,27	0,044
	G-H1	0,26	0,046
	Glucosepane	0,58	<0,0001*
Oxydation products			
	AASA	0,38	0,0029*
	Dityrosine	0,42	0,0009*
	GSA	0,36	0,0062*
	NFK	0,42	0,0011*

Nitration products

	3-NT	- 0,46	0,0003*
Other			

- 0,52 <0,0001\* CP

### Table 1

In vivo: Correlation between glucosepane and the histological score.

		Ν	Age (years)	Gender (M/F)	Glucosepane (nM)
	Control	29	34,4 ± 8,2	14/15	13,6 (10,1 – 18,1)
	eOA	28	43,3 ±13,3*	12/16	18,7 (13,3 – 35,5)* <sup>, 000</sup>
	aOA	38	70,7± 8,9***	15/23	76,3 (61,2 – 97,5)***
	Non-RA	32	51,7 ±18,1**	14/16	31,2 <b>(</b> 20,3– 45,2 <b>)</b> ** <sup>, 000</sup>
	eRA	35	60,4 ± 15,7***	13/22	46,1(31,1 – 77,8)*** <sup>,000</sup>
Table 3	•		_		

Human study: Plasma glucosepane is increased in patients with eOA and aOA.

Table 2 Correlation between glucosepane and Mach-1 parameters.

CONCLUSIONS. The glycation, oxidation and nitration of proteins are reactions related to the severity of osteoarthritis. The products of these reactions are measurable in blood by mass spectrometry and could be biomarkers of osteoarthritis. More specifically, glucosepane is an advanced glycation product very strongly increased in the severe form of the disease. In conclusion, serum glucosepane is a potential biomarker for diagnosis and progression of osteoarthritis.



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